Supplementary Information

Title

Mechanical perturbation of filamin A immunoglobulin repeats 20-21 reveals potential non-equilibrium mechanochemical partner binding function

Author names

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Distribution of unfolded contour length

For the unfolding of individual Ig repeats, the protein domain is unfolded to a polypeptide chain, which can be modelled by worm-like chain: $z/L = 1 - \sqrt{k_B T/4Af}$, where z is the unfolding step size, L is the contour length of the unfolded polypeptide, k_B is the Boltzmann constant, T is the absolute temperature, A is the persistence length of polypeptide which is ~0.5 nm, and f is force. From unfolding step size and unfolding force, the contour length can be obtained as shown in Figure S1. The distribution of control protein construct with only IgFLNa 21 in between of two handles of IgFLNa 1-3 shows one peak at ~31 nm, which is similar to the data from IgFLNa 1-8[1]. While for the protein construct with IgFLNa 20-21, two small peaks centred at 12 nm and -12 nm was observed.

Each amino acid contributes to contour length 0.4 nm. Therefore, the contour length of a single Ig domain with ~90 amino acids is around 36 nm. The contour length difference between unfolded poly-peptide and folded conformation with size of 4 nm is 32 nm. At 15 pN, the unfolding step size of a typical IgFLNa domain with ~90 amino acids is 20 nm, which is much longer than the observed ~8 nm unfolding step size.

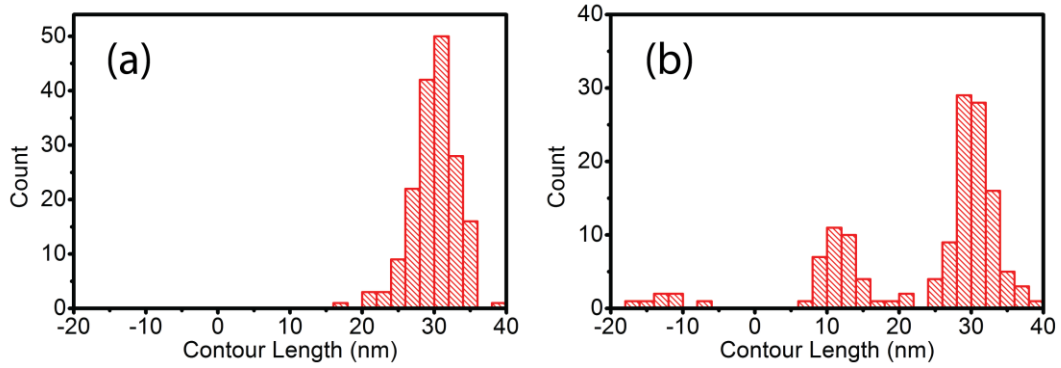

Figure S1 | Distribution of contour length of transitions. (a) The control protein construct was pulled with constant loading rate of 2.1 pN/second. (b) The protein construct with domain pair 20-21 was pulled with the same loading rate.

Force-dependent transition to U_2 state

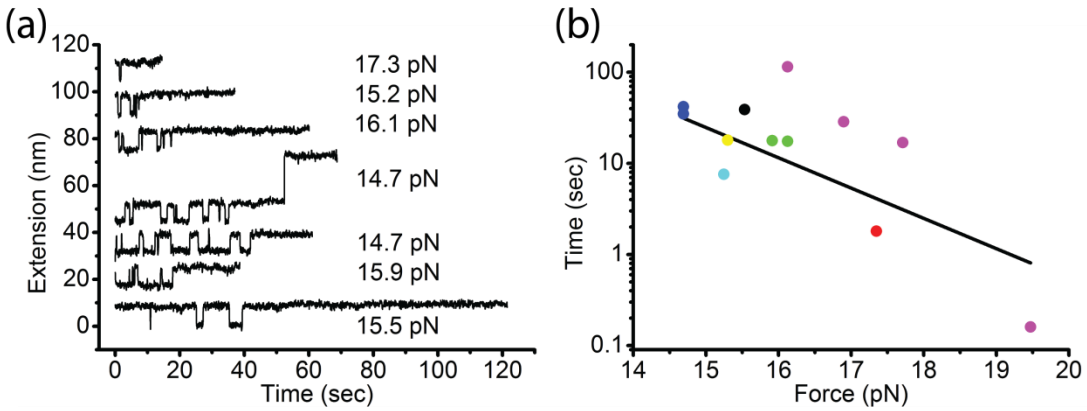

Figure S2 | Force-dependent selection between the two unfolded states U_1 and U_2. (a) Seven time traces were obtained from independent protein tethers of IgFLNa 20-21 at different forces. Each went through a region of rapid fluctuation between N and U_1, and followed by a region of stable long extension of U_2 state. The fourth time trace from the top contains a regular unfolding step of Ig domain in the handles of IgFLNa 1-3 or IgFLNa 21. (b) The duration τ of the region where rapid dynamic transitions between N and U_1 states occurred as a function of force. Symbols with the same color indicate data obtained from the same protein tether. These data were from the seven time traces in (a) and five additional time traces in the main text from Fig. 3 (a-b). The data is highly scattered due to 10% relative uncertainty in force calibration between different tethers as well as the stochastic nature of the selection between U_1 and U_2 at a particular force; regardless, an overall trend of shorter duration τ at larger forces is revealed.

Bimodal refolding kinetics observed at low loading rate

The bimodal folding kinetics of disrupted domain pair IgFLNa 20-21 can be observed under lower loading rate of 0.1 pN/second. Figure S3 shows the reversible (Fig. S3 a) and non-reversible (Fig. S3 b) folding processes in the same force-increasing scan (0.1 pN/second) and force-decreasing scan (-0.1 pN/second).

Once the IgFLNa 20 unfolds to the stable state and it cannot refold reversibly when force decreases. Even when force decreases to as slow as 2.4 pN with magnets moving away from the sample with constant speed (the force drops expentially), unfolded IgFLNa 20 still have chance to not fold successfully (Fig. S4), which can be determined by pulling the protein again to measure the extension and check if there is unfolding step.

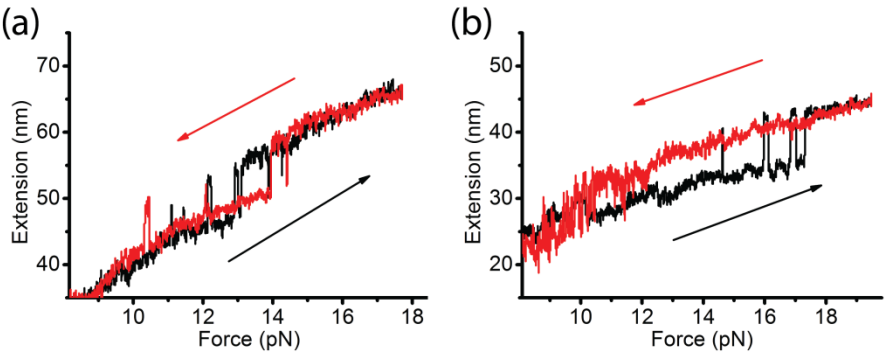

Figure S3 | Unfolding and refolding processes of protein construct with domain pair IgFLNa 20-21. Protein tether was stretched at a constant loading rate of 0.1 pN/sec in the force-increasing scan (black), followed by -0.1 pN/sec in the force-decreasing scan (red).

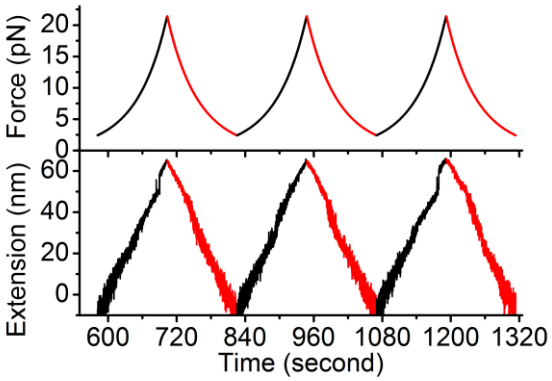

Figure S4 | The time course of force and measured extension of the protein. Magnets were moved to the sample and away from the sample with a constant speed of 20 um/sec to increase force and decrease force, respectively. In the three pulling cycles, unfolding signals of IgFLNa20 were detected in the first and third cycles, while no unfolding signal was detected in the second pulling cycle. Force was increased and decreased exponentially. It took ~25 seconds to increase force from 2.4 pN to 4 pN. Therefore, protein stays under force smaller than 4 pN for ~50 seconds between two pulling cycles.

DNA hairpin free energy landscape

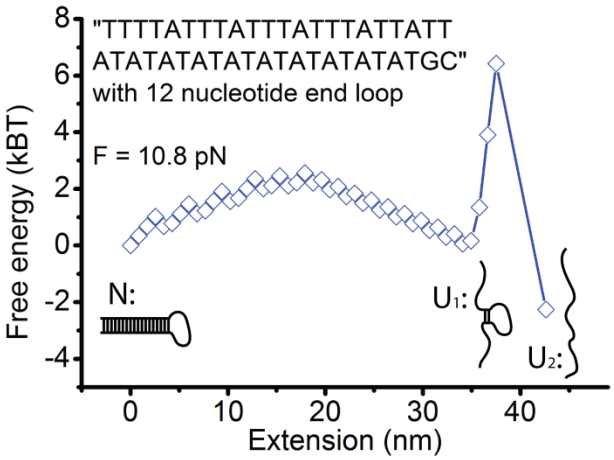

Figure S5 | The free energy landscape of a DNA hairpin containing a 12 nucleotide end-loop and specially designed stem sequence shown in the figure under stretching force of 10.8 pN. Folded state N and two unfolded states U_1 and U_2 are shown in the insert figures with minimal local free energy. In the intermediate unfolded state U_1, the end-loop remains; in the U_2 state, the loop is open and in a random coiled state. Using the SantaLucia DNA base pair stability data [2] and the single-stranded force response curve [3,4], the free energy was calculated as a function of the extension of the released single-stranded DNA. Overall, this gives a similar profile to our hypothesized model for unfolding of IgFLNa 20 in Fig. 5, despite the difference in the molecule size and the nature of the folding between IgFLNa 20 protein domain and DNA hairpin.

Magnetic tweezers setup

Vertical magnetic tweezers was built based on inverted microscope Olympus IX71. Oil immersion objective UPlanFLN from Olympus with 100X magnification and Numerical Aperture 1.3 was used to image beads. A piezo objective actuator P-725.CDD (PI, Germany) was used to move the objective vertically to change its focal plane with high accuracy. A half/half mirror was used to reflect light from white LED lamp to illuminate the sample through the objective. A Pike F-032B camera (Allied Vision Technologies, Germany) was used to capture images of the bead. Two magnetic rods were placed without gap in between to generate high force. A linear stage VT-40 (Micos, Germany) was used to move the magnets to control the force by changing the distance between the magnets and sample[5].

References

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